

The “other” Trace DNA – Forensic STR Typing of Ancient Human Remains and Environmental Samples left by Non-Human Primates

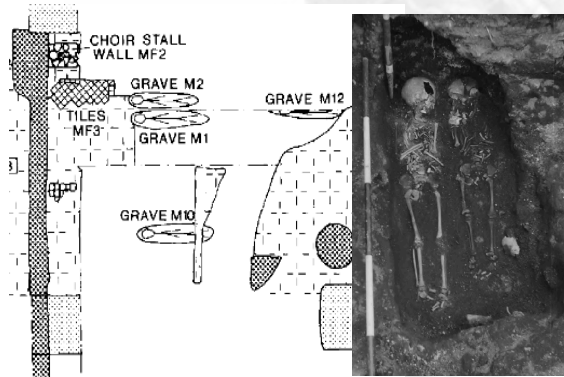
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Ancient DNA analysis of historical human remains explores similar questions, utilizing similar and frequently the same methodology as applied in the forensic human identification context, while doing so under extreme conditions regarding DNA content, degree of degradation and presence of inhibitors. Consequently, improvements of methods and procedures in one of the areas will inform the other and vice versa. Our current Ancient DNA based research utilizes historical skeletal human remains from the collection of the Museum of Gloucester to investigate a variety of methodological questions in both the osteological as well as the DNA analysis context.



Potential 16th century family burial inside the choir of a priory church: Successive burial layers. Upper layer: adult male and female M1 and M2, middle layer: adult male M3, bottom burials: 2 infants M4 and M5 (see picture, Ferris 2001).

Emma L Clemson: MSc Forensic Genetics & Human Identification

As in the forensic context, identification of individuals as well as kinship analysis involving group burials or across entire grave collectives are applications for DNA analysis-based methodology in the study of historical skeletal remains. The example here is a collective burial, excavated from the choir of a priory church in England, which based on the relative location of the individual remains indicates a potential family burial: The group includes five individuals which were buried in consecutive horizons (Ferris 2001).

Analysis here utilized an STR multiplex of own design based on published medium amplicon primer sets (Kimpton et al. 1993), combined with molecular sex determination (Amelogenin: Sullivan et al. 1993, SRY: Santos et al. 1997). In a second phase this was followed up using single-plex amplification of autosomal STRs (VWA, TH01), Y-chromosomal STRs (DYS19, DYS393: Roewer & Ellpen 1992, Kayser et al. 1997) and the two sexing loci. This was accompanied by osteological analysis of non-metric anatomical variants, which can be informative in the kinship analysis context (Ricaud et al. 2010).

For DNA analysis, ca. 1g of bone from rib- or minor long bone fragments was sampled, surfaces were removed, the sample decontaminated and ground to a fine powder. Initially, DNA was extracted from 0.1g bone following a Chelex protocol with subsequent precipitation (Schmerer in prep.) after a 70h decalcification in 0.5M EDTA (pH 8.3). Subsequent amplifications showed the presence of remaining inhibitors in extracts from the historical remains (Cant 2014), which is a common problem in ancient DNA analysis (Höss & Pääbo 1993, Schmerer et al 1999). Initially, ribs were used whenever possible to minimize invasiveness of sampling. To improve outcomes, the second phase utilized 0.3g of samples with higher content of compact bone, replacement of EDTA solution after 24h and decalcification for 96h (Schmerer 2003), followed by phenol chloroform extraction (Schmerer et al 1999, Schmerer 2003) and subsequent additional purification by silica column-based extraction (Omega Biotek 2013). Despite utilizing protocols optimized for the extraction of ancient DNA from historical human remains (Schmerer 2003) and additional adaptation of protocols (Hunt et al 2016), content of amplifiable DNA in extracts requires further improvement (Clemson 2016). Consequently, the next stage of the study will be based on more compact tissues like the femur diaphysis and roots of teeth, which commonly show a higher probability for preservation of the DNA contained.

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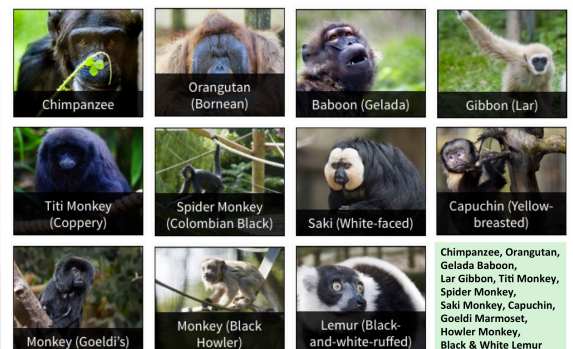
The second project focuses on the amplification of forensic STR loci from samples of non-human primates to enable DNA based identification and kinship analysis. Beyond this, it investigates the question at which evolutionary distance to humans – for which the markers in question were developed – amplification of forensic STR loci is still viable.

The study included samples from 11 primate species from phylogenetically close species like chimpanzees and orangutans to distant relatives such as Lemurs. Samples were collected at Dudley Zoological Gardens (West Midlands, UK).

As established in the primate behavior and conservation contexts, analysis are based on non-invasive sampling, namely fecal samples (Immel et al. 1999, 2000)

which contain traces of primate DNA from epithelial cells accumulated during the passage through the intestines of the individual (Albaugh et al. 1992). Material like this contains trace amounts of degraded DNA, while also resulting in substrate-based inhibition of PCR assays. even when utilizing protocols optimized for the extraction of DNA from primate faeces (Launhardt et al. 1998), as came obvious in initial attempts (Vanes 2016). The degree of inhibition differed dependent on species and consequently associated dietary composition. Based on these findings, the second phase of the study focused on optimization of substrate-specific protocols for sampling of cells from fecal specimen (external swab vs. internal sampling), DNA extraction and amplification, which resulted in improvement of amplification results (Vanes 2017).

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